In the United States Patent and Trademark Office

Serial Number: **10/663, 943**

Application Filed: **09/16/2003**

Applicant: **Rolland Hebert**

Application Title: **Diastereomers of S-adenosyl-l-methionine.**

Examiner/GAU: Lewis, Patrick T / 1623

Date faxed: 10.18.04

At: Seattle, WA 98102

DECLARATION UNDER RULE 132

Rule 132 Declaration regarding obviousness.

Assistant Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

Rolland Hebert declares as follows:

1. I am the inventor in the above patent application.

A. The current invention solves a problem that was never before recognized.

1. S-adenosyl-l-methionine is an important biological molecule participating in fundamental biological activities such as methylation and transulferation. Methylation activities are important in controlling expression of genes necessary not only for embryological development but also for controlling the expression of genes in the adult mammal. Hypomethylation of DNA and RNA leads to gene expression and their methylation leads to gene silencing. Such activation and silencing of genes has important biological and medical consequences both in the developing as well as the developed mammal. S-adenosyl-l-methionine is the principal methyl donor participating in methylation reactions in biological systems. S-adenosyl-l-methionine is a biologically unstable molecule and has been the subject of numerous attempts to stabilize it. S-adenosyl-l-methionine is unstable in two fundamental ways:

a. It is unstable due to intramolecular attack resulting in degradations products such as MTA. This problem with the degradation of S-adenosyl-l-methionine has by and large been resolved with cationic and anionic salts.

b. It is also unstable in terms of its diastereomeric configuration. (Applicant's current patent application discusses importance of isomers, enantiomers and diastereomers.) This problem has not been solved completely and S-adenosyl-l-methionine remains unstable in this regard. However, the Applicant's pending patent application addresses this diastereomeric instability by disclosing uses of the highest concentrations of the (S,S) S-adenosyl-l-methionine vs (R,S) S-adenosyl-l-methionine diastereomers. Only now are there methodologies available to arrest somewhat the epimerization from (S,S) S-adenosyl-l-methionine to (R,S) S-adenosyl-l-methionine diastereomeric configuration of this important molecule.

2. The (S,S) S-adenosyl-l-methionine configuration is the form that participates in the methylation reactions. The **(R,S) S-adenosyl-l-methionine** configuration is a potent

methyltransferase inhibitor and thus it exactly inhibits the important reaction for which (S,S) S-adenosyl-l-methionine is so vital, namely, methylation.

3. Borchardt and Wu in a paper entitled " Potential Inhibitors of S-adenosyl-l-methionine-dependent methyltransferases. 5. Role of the Asymmetric Sulfonium Pole in the Enzymatic Binding of Adenosyl-l-methionine", Journal of Medicinal Chemistry, 1976, Vol. 19, No. 9, pp 1099-1103, and included in this declaration as **Exhibit A**, report that the **(+)-L-SAM** (nomenclature no longer used for **(R,S)-S-adenosyl-l-methionine)** is a potent **inhibitor** of enzyme-catalyzed transmethylation reactions. Please consult in particular table 1 page 1102.

4. (+)-L-SAM, that is, **(R,S)-S-adenosyl-l-methionine**, is as potent an inhibitor of S-adenosyl-l-methionine-dependent transmethylation reactions as is SAH, S-adenosyl-homocysteine. The fact that (R,S)-S-adenosyl-l-methionine is as potent an inhibitor of SAM-dependent transmethylation reactions as S-adenosyl-homocysteine was never seen as having important clinical implications since at the time the paper was written the dangers of SAH or homocysteine (and its ability to inhibit SAM-dependent transmethylation reactions, in particular, COMT) were not recognized and therefore not understood. The dangers associated with S-adenosylhomocysteine are attributed to its ability to inhibit SAM-dependent transmethylation reactions that are implicated in hundreds of biochemical reactions ranging from synthesis of creatine in the liver, to protein and gene methylation. Please consult in particular p 1103, paragraphs 1 and 2 of first column for the discussion of the S-adenosyl-l-methionine-dependent transmethylation inhibition properties of (+)-L-SAM. Additionally, the epimeric instability of the S-adenosyl-l-methionine molecule in powder form was not recognized and never addressed in this paper.

5. However, since the Borchardt and Wu paper was written, SAH and elevated serum homocysteine levels have been recognized as independent risk factors for a number of pathological conditions. Conditions associated with elevated serum homocysteine levels (which result in increases in serum S-adenosylhomocysteine and then increased intracellular S-adenosylhomocysteine levels) are the following: central nervous system demyelination, reduced neurotransmittor synthesis, decreased chemotaxis and macrophage phagocytosis, altered membrane phospholipid composition and membrane fluidity, altered gene expression, and cell differentiation. Reference: Ping Yi et al "Increase in Plasma Homocysteine Associated with Parallel increases in Plasma S-adenosylhomocysteine and lymphocyte DNA hypomethylation", The Journal of Biological Chemistry, Vol 275, No. 38, September 22, pp 29318-29323, 2000, included in this declaration as **Exhibit B**.

6. Detich et al, in a paper entitled "The methyl donor S-adenosylmethionine inhibits active demethylation of DNA: a candidate novel mechanism for the pharmacological effects of S-adenosylmethionine", J. Biol.Chem., 2003, June 6; 278 (23) : 208812-20 and included in this declaration as **Exhibit C**, show the potential tumor protective mechanism of S-adenosyl-l-methionine and the importance of intracellular S-adenosyl-l-methionine concentrations in cancer prevention. This mechanism is due to the ability of (S,S) S-adenosyl-l-methionine to reverse or correct DNA hypomethylation.

7. Hypomethylation of the DNA is a hallmark of cancer cells and the correction of this hypomethylation leads to proper gene expression and reversal or prevention of cancer. In this paper Detich warns that since hypomethylation of the DNA may be a causal factor in tumorigenesis, one must be very cautious in developing drugs that inhibit DNA methylation since they may cause further hypomethylation of the DNA. There are currently new drugs on the horizon that inhibit DNA methylation thus targeting islands of

the genome that exhibit hypermethylation as one methodology to treat cancer. However, as Detich and others have warned, using new drugs such as anti-sense drugs or even 5-azacytidine which is known as a potent inhibitor of DNA methylation, may lead to further cancers by causing genome wide hypomethylation.

8. Gaudet et al, in a paper entitled "Induction of Tumors in Mice by Genomic Hypomethylation", Science Vol 30, 18 th April , 2003, pp 489-492, and included in this declaration as **Exhibit D**, report that genomic hypomethylation caused tumorigenesis in mice. They note in the conclusion on page 492, first column, paragraph three, that drugs such as 5-azacytidine may be considered double-edge swords since while they are able to cause hypomethylation of the hypermethylated islands of the genome and treat some types of cancer, they risk causing global genomic hypomethylation. This global hypomethylation of the genome may lead to an increased risk of cancer in other tissues. The above two papers argue against the use of drugs that would lead to DNA hypomethylation. The use of **(R,S) S-adenosyl-l-methionine** in high percentages in a S-adenosyl-l-methionine drug should be avoided due to its ability **to inhibit S-adenosyl-l-methionine-dependent transmethylation reactions**.

Current status and stability of S-adenosyl-l-methionine

9. S-adenosyl-l-methionine is currently only available commercially as a mixture of (S,S)-S-adenosyl-l-methionine and (R,S)-S-adenosyl-l-methionine. Since it is very unstable during the fermentation and purification process, the molecule is sold in an (80%:20%) ratio by weight of (S,S)-S-adenosyl-l-methionine to (R,S)-S-adenosyl-l-methionine respectively (and often much less than this percentage of (S,S)). However, it is now known that this ratio of (S,S)-S-adenosyl-l-methionine to (R,S)-S-adenosyl-l-methionine, while starting out at (80%:20%) after the purification process, begins to

change almost immediately. That is, S-adenosyl-l-methionine is subject to a chemical change called **epimerization** that results in a change in configuration of the S-adenosyl-l-methionine molecule from its desired (S,S) S-adenosyl-l-methionine conformation to its undesirable (R,S) S-adenosyl-l-methionine conformation while on the shelf.

10. The instability of the S-adenosyl-l-methionine diastereomeric forms in solution had been known for some time. However, its instability in terms of its diastereomeric forms (that is, their epimerization from (S,S) S-adenosyl-l-methionine conformation to (R,S) S-adenosyl-l-methionine conformation in the powdered state, had not been known. **Gennari US 4,465, 672** was published in 1984. It appears that Gennari did not know about the epimeric or diastereomeric instability of the S-adenosyl-l-methionine molecule in powdered form and he did not discuss the epimeric instability of the S-adenosyl-l-methionine molecule.

B. Up to now, those skilled in the art never appreciated the advantage of the invention although it is inherent.

1. In fact, there have been no scientific publications or patent publications to the Applicant's knowledge, pointing out the epimeric or diastereomeric instability of the S-adenosyl-l-methionine molecule in powdered form until very recently. Berna et al in United States Patent Application 20020010147, January 24, 2002, show for the first time in example 4 how unstable commercially available (for 2002) S-adenosyl-l-methionine is. (The Applicant, in an IDS for USPTO application number 09/943243 (sent 12.17.01) related to this current pending patent application 10/663,943, provided the USPTO with a copy of the Berna application WO 01/90130 A1.)

2. In WO 01/90130 A1, Berna et al show for the first time that the S-adenosyl-l-methionine diastereomer mixture as a powder is very unstable over time. Please consult pages 9 and 10 of the Berna application WO 01/90130 A1 in which a table under example 4 shows the instability of a commercial product of S-adenosyl-l-methionine called SAMIR. SAMIR contains 58% (S,S) S-adenosyl-l-methionine and 42% (R,S) S-adenosyl-l-methionine. It is known that the process for the production of S-adenosyl-l-methionine yields 80% (S,S) S-adenosyl-l-methionine and 20% (R,S) S-adenosyl-l-methionine immediately after production and the epimerization takes place on the shelf over time.

3. Berna et al do not discuss the potential negative clinical impact of the methyltransferase inhibition activity of the (R,S) S-adenosyl-l-methionine diastereomer since they do not recognize the significance that methyltransferase inhibition would have on the genome or on other S-adenosyl-methionine dependent methyltransferase reactions. In fact, on page 2 of the Berna patent application WO 01/90130 A1, Berna et al have stated that according to De La Haba et al, only the (S,S)- S-adenosyl-l-methionine is active for the transmethylation reaction. However, they do not report or apparently recognize that the (R,S) S-adenosyl-l-methionine form has important negative biological activity, that is, inhibition of the precise reaction for which the (S,S) S-adenosyl-l-methionine is required. Clearly, even at the late date of the Berna application, (priority date 25 May 2000), those skilled in the art did not know about the precise reason for which the (R,S) S-adenosyl-l-methionine diastereomer should be separated from the (S,S) S-adenosyl-l-methionine diastereomer.

4. Further, although Berna et al would be considered to be skilled in this particular art, they do not disclose a method for the use of the (S,S) S-adenosyl-l-methionine salts, only the process for their manufacture. The Applicant's pending patent

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. 1001 and that such willful false statements may jeopardize the validity of the application of any patent issued thereon.

Very respectfully,

RHebert date 10.18.04

Rolland Hebert, Applicant Pro Se
427 Bellevue Ave. E. Suite 301
Seattle, WA 98102
Cell phone 206.650.4489
Customer number 29133

Enclosures; **EXHIBITS A-F**

CERTIFICATE OF FACSIMILE TRANSMISSION

I hereby certify that this correspondence and attachments, if any, will be faxed to GAU 1623 of the US Patent and Trademark Office at 703-872-9306 on the date below.

Date: 10.18.04

Inventor's Signature RHebert 10/18/04

Rolland Hebert

application is thus the first to teach the use of the (S,S)- S-adenosyl-l-methionine/ (R,S)-S-adenosyl-l-methionine in the concentrations indicated in the application for the uses indicated. Please find the Berna patent application WO 01/90130 A1 included in this declaration as **Exhibit E.**

C. Lack of implementation: If the invention were in fact obvious, because of its advantages, those skilled in the art surely would have implemented it by now. Thus, the fact that those who are skilled in the art have not implemented the current invention, despite its great advantages, indicates that it is not obvious.

1. To date, there has been no implementation of the methods that are the subject of the current pending invention, that is, no one has anticipated the use of the particular percentages of (S,S) vs (R,S) S-adenosyl-l-methionine as stated in the current invention for the uses indicated in the pending patent application.

2. Thus, in view of the distinct advantages of the current invention, it would seem that those skilled in the art surely would have implemented it by now. Indeed, for drug development, current FDA guidelines require that diastereomers of drugs be separated and tested individually. For rational drug development of S-adenosyl-l-methionine in the US, it is required that the diastereomers be separated and evaluated separately and show no untoward side effects. Please see Federal Register/Vol 65, No. 251/ Friday, December 29,2000/ Notices column 2 3.3.1 (d) for a discussion of isomers and epimers included in this declaration as **Exhibit F.** The (R,S) S-adenosyl-l-methionine diastereomer must be separated from or exist in such small percentage in relation to the (S,S) S-adenosyl-l-methionine diastereomer for the reasons discussed above. To date, there has been no attempt to develop an (S,S)/(R,S) S-adenosyl-l-methionine diastereomeric drug as discussed in the Applicant's patent application.